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Supplemental Materials

for

Introducing Mammalian Cell Culture and Cell Viability Techniques in the Undergraduate Biology Laboratory

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Appendix 1: Student handout – non-biology majors.

General Education Biology Laboratory

An Introduction to Mammalian Cell Culture

Objectives of the lab:

This week's lab is designed to introduce you to mammalian cell culture concepts. Upon completion of this lab, you should be able to:

1) Passage mammalian cells
2) Count cells using a cell counting chamber
3) Calculate cell concentrations
4) Determine cell viability
5) Create a graph of your data

Introduction to HeLa Cells

The HeLa cell line was established in 1951 from a biopsy of a cervical tumor taken from Ms. Henrietta Lacks, a working-class African-American woman living near Baltimore. The cells were taken without the knowledge or permission of her or her family, and they became the first human cells to grow well in a lab. They contributed to the development of a polio vaccine, the discovery of human telomerase and countless other advances. A PubMed search for 'HeLa' turns up more than 75,000 papers. "My lab is growing HeLa cells today," Collins told *Nature* in an interview on the NIH campus in Bethesda, Maryland. "We're using them for all kinds of gene-expression experiments, as is almost every molecular-biology lab." (Excerpt from E. Callaway, *Nature* 2013, doi:10.1038/500132a)

Harvesting Cells:

1) Remove the culture media from your petri dish using a bulb pipette. Add 5ml of the 1xPBS solution, gently rock the plate, and remove the 1xPBS solution with a bulb pipette. Add 1 ml trypsin solution (cleaves proteins attaching cells to the plate) and gently rock the plate so that all cells are covered.

2) Incubate the plate for at least 10 min with occasional rocking. Cells should start to release from the plate before you begin the next step.

3) Wash cells with 1 ml of 1xPBS/1%FBS to neutralize the proteolytic activity of trypsin. Transfer the 2 ml cell suspension to a blue cap conical tube. Add 8 ml of 1xPBS to the conical tube so that the final volume of cell suspension is approximately 10 ml.

4) Cap the tube; invert 2-3x

Experimental Procedure:

Several factors can impact cell survival rates including temperature, pH, salt, and drug exposure. In this lab, you and your partner will investigate the effects of three different temperature environments (4°C, 37°C, and 42°C) on cell survivability.

1) Set up 9 individual microcentrifuge tubes. Remember that we will run 3 replicates per treatment group so label each tube with the appropriate temperature treatment.

2) Aliquot 500 ul of cell suspension per tube.

3) Based on what you know about temperature and cell survival develop a hypothesis as to what the treatment will do to the cells and explain why this may happen on a cellular level.

4) Incubate your vials at the appropriate temperature for 30 minutes. During the incubation time, you may want to set up you cell number calculations.

During this incubation period you should also practice staining cells with trypan blue and counting them using grid slides. You will be calculating the concentration of live cells in the cell suspension you just generated.

- Transfer 20 ul of the cell suspension (from the 15 mL conical tube) to a microcentrifuge tube.
- Add 20 ul of trypan blue to the microcentrifuge tube. Trypan blue is vital dye that is absorbed by dead cells and excluded by living cells. Load 10ul of cell/dye mixture into a single chamber on the **counting slide**.
- Note that:

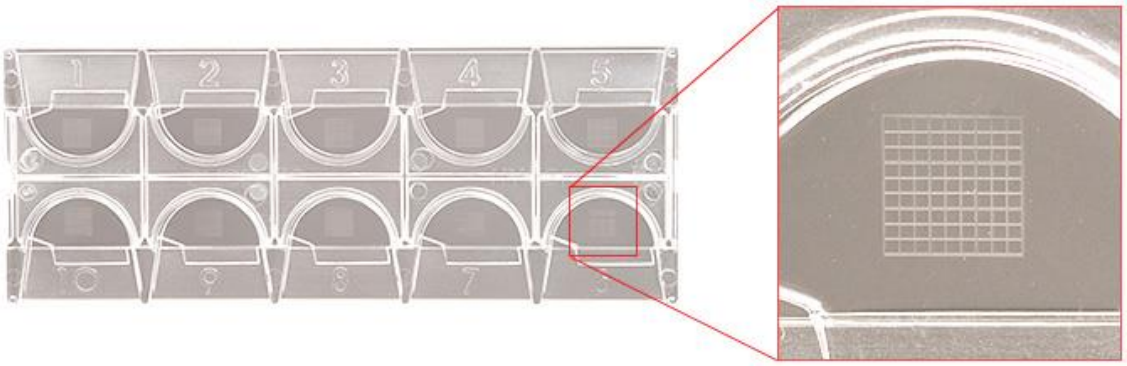
    a. Dark blue cells = Dead cells
    b. Light blue cells = Living Cells

Use the following formula to calculate cell concentration:

(#Live Cells/#Small Squares)*7500*Trypan Blue Dilution = # cells/ml

Example: If you diluted your cells 1:1 in trypan blue, counted 90 cells in 18 squares then the equation would be:

(90/18)*7500*2 = 75,000/ml = 7.5×10^4 cells/ml



Record your data below:

# of cells counted: _____

# of squares: _____

Trypan Blue dilution: _____

Cell concentration of original cell suspension: _____

Once the 30-minute incubation period is over, continue to follow the protocol below.

5) Use trypan blue dye as before to count the cells, but, this time, record both the number of live and dead cells. Calculate the proportion of live cells using the following equation:

Total # Live Cells/ Total # of Cells (live and dead)*100 = Cell Viability (%)

6) Record your data in the table below.

Treatment	Replicate 1	Replicate 2	Replicate 3	Average

7) Using Microsoft Excel software, generate a graph. Make sure your graph has a (1) title, (2) axes labels, (3) and a figure legend. Your figure legend should include a description of the results, the hypothesis being tested and comments regarding whether or not the data resulted your hypothesis being accepted or rejected.

Appendix 2: Sample student analysis – non-biology majors.

General Biology Education Sample Student-Generated Figure and Analysis

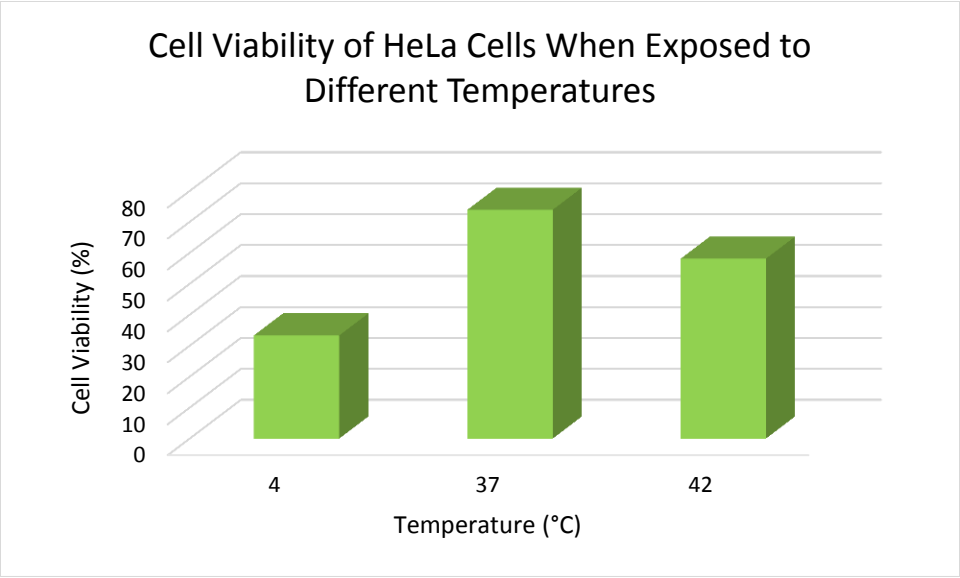


Figure 1. Comparison of HeLa cell viability based on temperature.

This experiment was testing how well cells can survive in different temperatures of incubation. The graph above shows the results, with the most cells surviving in a temperature of 37°C and the survival rate decreasing as the temperatures move away from 37°C. The further away from 37°C, the lower the survival rate as evidence by the fact that less cells survived at 4°C than at 42°C, while both had a lower survival rate than at 37°C. The hypothesis for this experiment was that, as the temperature moved away from our internal body temperature of 37°C, less cells would be able to survive. The data in the chart above gives evidence that our hypothesis should be accepted, as cells cannot survive as well in temperatures that are not our internal body temperature (37°C).

Appendix 3: Student handout – biology majors.

General Biology Laboratory

An Introduction to Microscopy and Mammalian Cell Culture

Introduction:
This week's lab is designed to introduce you to compound light microscopy and mammalian cell culture concepts. At the end of lab you should be able to:

1) Use a compound light microscope
2) Manipulate mammalian cells in a non-sterile environment
3) Calculate cell concentrations
4) Develop a hypothesis as to what a given treatment does to a human cell
5) Determine cell viability
6) Create a table of your data and methods section

PART A: Microscopy[1]

I. Care and Use of the Compound Microscopes

A compound microscope can magnify from 40 to 2000 times (40 - 2000 X). Microscope quality depends, however, on resolving power and not so much on magnification. Resolving power is the ability to distinguish between two points in the field of view. Thus, if you can magnify 1000-fold yet cannot resolve detail, your microscope would be of little value. Even more important may be the abilities of the microscopist to learn the capabilities of his or her microscope and to gain proficiency in the use of the instrument.

Do's and Don'ts

1. Always carry a microscope with both hands, one grasping the handhold in the back and one grasping the bottom.

2. Do not swing the microscope at arms length and do not bang it onto the bench top.

3. Never place the microscope near the bench's edge and keep electric cords out of the way, towards the center of the bench.

4. Examine your material first using the lower power objective (i.e. 4X); then use a higher power objective (i.e. 10X or 40X). Replace and remove a slide only after the lowest power objective has been rotated into viewing position.

5. Never attempt to repair a microscope or force an adjustment knob. You may severely damage the instrument.

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[1] Adapted from "Using Microscopes" by Dr. Malcolm Campbell (2000); Department of Biology, Davidson College, Davidson, NC 28036

II. Parts of a Microscope

Find an image of a compound microscope to help identify the following components.

Ocular: The piece you look through. Sometimes called an ocular lens or eyepiece, this unit is really a series of lenses. Our microscopes are binocular, having two oculars. Learn to use both eyes; focus your eyes as if you were looking at an object about five to ten meters in front of you. You should adjust the width of the oculars to match the width of your eyes.

Objective lens: Sometimes called the objective; a set of self-contained lenses. The objective gathers light from the specimen and directs it through the tube to the oculars. These scopes have three objectives (4X, 10X, and 40X).

Nosepiece: The rotating turret to which objectives are mounted. There are preset positions for each objective, detected by slight pressure changes while turning the nosepiece and usually a clicking noise. You should not grab the objectives to turn the nosepiece - use the black ring instead.

Stage: The flat surface upon which slides are placed. On your microscopes, the stage moves up and down and the slide is manipulated by a geared device. A moveable stage is sometimes called a mechanical stage. The slide is moved left/right and front/back by two knobs projecting downward from the stage.

Condenser: A lens system under the stage that gathers light from the light source and focuses it on the specimen. There is a diaphragm in one part of the condenser that can be adjusted to allow the viewer to see different parts of the cell when using bright field illumination. You should experiment with this control.

Condenser Adjustment Control: Under the stage on the left side is a small knob that is used to adjust the height of the condenser. For the most part, this will always be all the way up.

Light Switch Control: The light switch and intensity controls are on the right side of the microscope base, about half way up the side. There is an on/off switch as well as a brightness control. Use only as much light as necessary to illuminate the specimen.

Light Source: On our microscope the light source is built into the base and is directly under the condenser.

Adjustment (Focus) Knobs: Both coarse (large) and fine (small, inner) adjustment knobs are found on both sides of our microscopes. Remember that the coarse adjustment is used only with the low-power objective. These control a gear mechanism that raises and lowers the stage.

Different Types of Microscopy: Bright Field, Dark Field, and Phase-Contrast

The type of illumination that we will be using with our microscopes is called **bright field**. Think of the light source as producing a solid tube of light that travels up to and through the condenser. When you view specimens with all of this light, you are using bright field illumination.

Dark Field (not used in today's lab with our microscopes): Dark field illumination seems like an oxymoron, but in this case it describes an unusual way of viewing specimens in some compound microscopes. The light that passes directly through the condenser does not enter the objective lens. Only light that has been scattered or reflected by the specimen enters the objective. As a result, you wind up seeing bright objects on a dark background.

Phase-Contrast (not used in today's lab with our microscopes): Phase-contrast microscopy allows us to see otherwise transparent organelles and structures. In a phase-contrast scope, the light hits the specimen and some of the light continues in a direct path. Other portions of the light pass through membranes which redirect the light. This redirected light is slowed down by 1/4 a wavelength (a phase shift of 1/4) by passing through a special filter. This special filter is shaped like a doughnut and is called a phase ring. The redirected and out of phase light eventually reaches your eyes but not at the same time as the unaltered light that passed straight through. The end result is that you can see transparent structures because they altered the pathway of light as it went through the structures. This allows us to view subcellular structures within living cells.

III. Viewing a Specimen:

Every time you work with a microscope:

1) Position the scope so it is directly in front of you and your chair is adjusted so that you do not have to strain to view a specimen.

2) Make sure the light intensity control is turned all the way off before turning on the microscope.

3) Make sure the 4X objective is in place over the specimen.

4) Switch on the light source and then dial the adjustment knob to a level that is comfortable for your eyes. Start with the stage in the lowest position and place the slide in the slide holder.

5) Use the coarse adjustment to raise the stage slowly while looking through the oculars until the specimen comes into focus. Adjust the focus to its sharpest with the fine adjustment knob.

6) Readjust the light intensity to reduce glare and center the specimen in the field of view by moving the stage.

7) Place the 10X objective over the specimen and sharpen the focus with the fine adjustment knob (only!) as necessary.

8) Adjust the condenser's diaphragm to maximize the resolution of the structure you are trying to see. The actual setting will depend on what you are trying to see. Small translucent objects will be seen more easily with the diaphragm closed substantially while large pigmented structures are easier to see with the diaphragm wide open.

9) Repeat steps 8 and 9 but use the 40X objective instead of the 10X.

Cells:

The HeLa cell line was established in 1951 from a biopsy of a cervical tumor taken from Henrietta Lacks, a working-class African-American woman living near Baltimore. As was common practice at the time, the cells were taken without the knowledge or permission of her or her family. These tumor cells were the first human cells that grew and divided continually in a lab. They contributed to the development of a polio vaccine, the discovery of human telomerase and countless other advances. A PubMed search for 'HeLa' turns up more than 75,000 papers. "My lab is growing HeLa cells today," Collins told *Nature* in an interview on the NIH campus in Bethesda, Maryland. "We're using them for all kinds of gene-expression experiments, as is almost every molecular-biology lab." (Excerpt from E. Callaway, *Nature* 2013, doi:10.1038/500132a)

Preliminary Data:

1) Remove the culture media from your petri dish using a bulb pipette. Add 5ml of the 1xPBS solution, gently rock the plate, and remove the 1xPBS solution with a bulb pipette. Add 1ml Trypsin solution (cleaves proteins attaching cells to the plate) and gently rock the plate so that all cells are covered.

2) Incubate the plate for at least 10min with occasional rocking. Cells should start to release from the plate before moving to the next step.

3) Wash cells with 1ml of 1xPBS/1%FBS to neutralize the proteolytic activity of trypsin. Transfer the 2ml cell suspension to a blue cap conical tube. Add 8ml of 1xPBS to the conical tube so that the final volume of cell suspension is approximately 10ml.

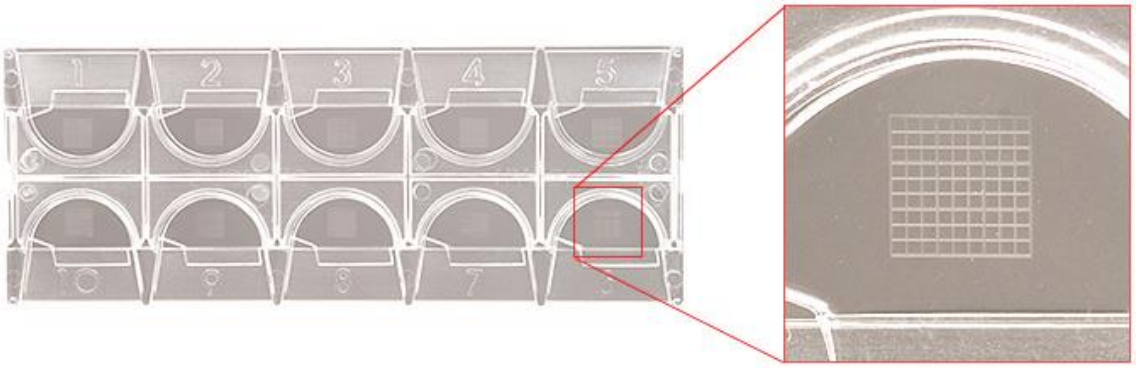
4) Cap the tube, invert 2-3x and transfer 20ul of the cell suspension to a microcentrifuge tube. Add 20ul of Trypan blue to the microcentrifuge tube. Trypan blue is vital dye that is absorbed by dead cells and excluded by living cells. Load 10ul of cell/dye mixture into a single chamber on the counting slide.
    a. Dark blue cells = Dead cells
    b. Light blue cells = Living Cells

Use the following formula to calculate cell concentration:

(#Cells/ #Small Squares)*7500*Trypan Blue Dilution = #Cells/ml

Example: If you diluted your cells 1:1 in trypan blue, counted 90 cells in 18 squares then the equation would be:

(90/18)*7500*2 = 75,000/ml = 7.5e4/ml



Record your data below:

Experimental Data:

Several factors can impact cell survival rates including temperature, pH, salt, and drug exposure. You and your partner should decide on one treatment with at least 3 different concentrations and measure cell survivability. Identify which one you would like to test and formulate a hypothesis. When designing your experiment, make sure to include a **negative control**. Record your hypothesis AND draw a graph of what you expect your data to look like in your lab binder.

1) Make a 10ml cell suspension in 1xPBS using the cell concentration that you just calculated, so that the suspension contains $4x10^4$ cells per ml. This will be the cell suspension that you use in your next experiment. If you do not have enough cells to make a 10ml $4x10^4$ cell/ml solution, then use all of your cells to make the 10ml suspension and record the concentration. Set up 12 individual microcentrifuge tubes. Aliquot 500ul of cell suspension per tube. Remember that you need to use 3 replicates per treatment.

2) Based on the cell treatments offered by your instructor, develop a hypothesis as to what the treatment will do to the cells. Try to come up with a justification as to why this may happen.

3) Design a protocol for testing your hypothesis. Keep the following in mind: control variables, sample size, and data collection. Make sure to check with your instructor as you may need to adjust your experiment for the available materials. Once your protocol has been approved, conduct your experiment.

   Example Experimental Layout (you and your partner will determine the treatment, concentration, and exposure times). Incubate cells with chosen treatment for at least 30min.

   Microcentrifuge tubes

   a. Negative Control (x3) use 500ul of 1xPBS
   b. Treatment? (x3) use 500ul
   c. Treatment? (x3) use 500ul
   d. Treatment? (x3) use 500ul

4) Use Trypan blue dye as before and calculate the number of live cells in each of the samples. Record your data in a table in Excel. Make a graph that represents the results. Make sure to include appropriate statistics in your graph. You will turn in this graph along with a detailed methods section in to your instructor.

Appendix 4: Sample student analysis – biology majors.

Biology Major Sample Student-Generated Figure, Methods, and Results Analysis

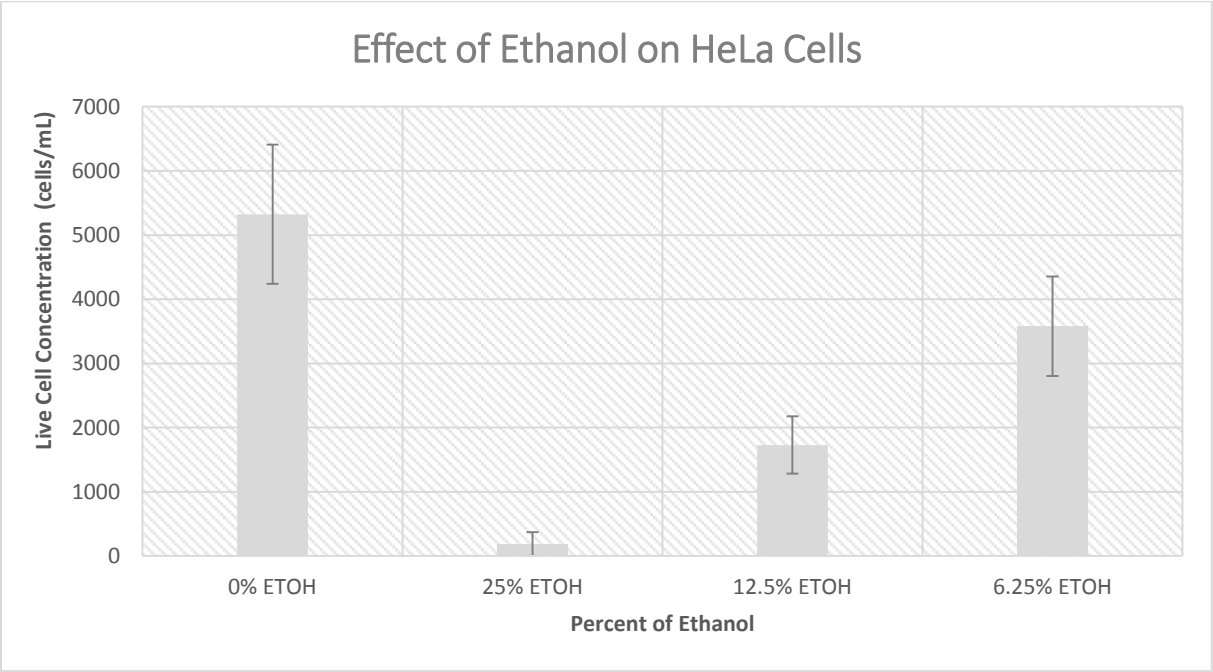


Figure 1. Comparison of HeLa cell viability based on ethanol concentration.

Methods

The HeLa cells were given to us in a petri dish filled with culture media. We removed the culture media with a bulb pipette and added 5 mL of of 1xPBS solution and gently rocked it around the dish. After removing the 1xPBS solution witha bulb pipette, we added 1 mL of Trypsin solution in order to break up the cells from each other and from the bottom of the dish. We allowed the dish to incubate for 10 min while occasionally rocking it to insure that the cells would start to release from the dish. 1 mL of 1xPBS/1%FBS was added to neutralize the proteolytic activity of the trypsin. All of the liquid in the dish (2 mL total at this point: 1 mL trypsin and 1 mL of 1xPBS/1%FBS) was transferred to a blue capped conical tube and 3 mL of 1xPBS was added so that the final volume was 5 mL. After inverting the tube a few times to make sure the cells were evenly distributed, we transfered 20 ul of the cell suspension to a microcentrifuge tube and added 20 ul of trypan blue (dye which is absorbed by dead cells which allows for the counting of live cells). We loaded 10 ul of the cell/ dye mixture into a chamber of the counting slide and counted the 4 corner squares and the middle square (5 total squares). We used the formula, (#Cells/ #Small Squares)*7500*Trypan Blue Dilution = #Cells/mL, to find the initial cell concentration. The trypan blue dilution in our case was 1:1 so "2" was the value and the number of squares was 81. We then created an experiment in which we added 3 treatments of different concentrations of ethanol in order to test the effect of ethanol on HeLa cells. We labeled 12 microcentrifuge tubes since we had 4 treatments (0% EtOH, 6.25% EtOH, 12.5% EtOH, and 25% EtOH) and conducted 3 trials for each. We placed 250 ul of cell suspension in all 3 25% tubes and filled the remainder with 250 ul of 50% EtOH in order to make the 25% ethanol. In the 12.5% tubes, we placed 125 ul of 50% EtOH, 125 ul of PBS, and 250 ul of cells. In the 6.25% tubes, we placed 62.5 ul of EtOH, 187.50 ul of PBS, and 250 ul of cells. In the 0% tubes, we placed 250 ul of cells and 250 ul of PBS. After letting incubate for 30 min, we did as before and took 20 ul of each tube's contents and 20 ul of trypan blue and loaded the single chambers of the counting slide and proceeded to count the cells and used the equation to find the cell concentration.

Results

As we chose to experiment with different concentrations of ethanol, our hypothesis was that as the ethanol concentration increases, it will cause the HeLa cells to die. Our negative control was the 0% ethanol which was basically the initial concentration although we conducted it 3 more times for the experimental data. Our hypothesis was proven to be supported by our data because as we increased the ethanol concentration, the HeLa cells began dying quickly. Our T Tests also showed that we accpeted 2 of our null hypothesis' which was between 0% & 6.25% and 6.25% & 12.5%. The T Tests for these 2 pairs came out higher than .05 which allowed for us to accept the null hypothesis which means there is no pattern present between the 2.

Appendix 5: Instructor preparation guide.

An Introduction to Mammalian Cell Culture

Instructor's Preparation Guide

Table of Contents

Summary

In this exercise, students will compare the viability of mammalian cells growing in culture after exposure to different treatments. Students will be asked 1) to develop a hypothesis about the relationship between mammalian cell survival and a particular chemical or environment and 2) to make predictions as to how HeLa cell survivorship will be affected by manipulating those chemicals or environment. Students will first disperse adherent HeLa cells from their culture dish and calculate an initial concentration of living cells in their culture by staining them with trypan blue. Students will then carry out their experiment with replicates to test their hypothesis and will present their post-exposure HeLa cell survivorship data in a graph with a figure legend. This exercise was originally designed for a 3-hour class of 18 students working in pairs, but could easily be expanded to laboratory sections with additional students.

Laboratory Objectives

This exercise is designed to introduce students to the concept of mammalian cell culture and to reinforce the concepts of experimental design and microscopy. In this exercise, students will:

- Manipulate mammalian cells in a non-sterile environment
- Calculate the concentration of mammalian cells in a culture
- Assess the impact of different treatments on cell survival rates
- Generate a graph and figure legend that represents their data

Anticipated Timelines

Preparation

Initiate primary HeLa cell culture: 6 – 10 days in advance of the exercise

Pass HeLa cell subcultures in dishes: 3 – 5 days in advance of the exercise

Prepare all solutions and set out supplies: the day of the exercise

Procedure for General Education Biology (non-majors) – 2 hours

Disperse monolayer of HeLa cells from petri culture dish …… 15 minutes

Suspend HeLa cells and prepare replicates for each treatment …….. 5 minutes

Incubate replicate HeLa cell suspensions at 4°C, 37°, or 42°C …… 30 minutes

Develop a hypothesis and make predictions about survival …… concurrent

Count living HeLa cells in original suspension using trypan blue ……. concurrent

Further discuss the history of HeLa cells and ethics of their use in research …….. concurrent

Count living HeLa cells in replicate suspensions using trypan blue …… 45 minutes

Prepare graph and corresponding figure legend …….. outside of class

Procedure for Biology Majors – 3 hours

Develop a hypothesis and make predictions about survival …….. 15 minutes

Disperse monolayer of HeLa cells from petri culture dish …… 15 minutes

Suspend HeLa cells and count living HeLa cells using trypan blue …….. 15 minutes

Prepare a suspension with 4 x 10^4 cells / mL and set up replicates …… 15 minutes

Expose replicate HeLa cell suspensions to treatments …… 30 minutes

Count living HeLa cells in replicate suspensions using trypan blue …… 60 minutes

Prepare graph and corresponding figure legend …….. outside of lab

Reagents, Supplies and Equipment

The following tables outline the reagents, supplies and equipment needed to conduct this laboratory exercise with a class of 18 – 24 students working in pairs. If ordering for multiple laboratory sections, check whether bulk purchasing options are available for each item, as this may further reduce the unit price. While the tables denote preferred vendors for each item, the prices listed do not reflect contract pricing extended to different institutions; suitable alternate vendors / items may be used. The prices listed also do not reflect potential shipping, handling, cold pack, dry ice and freight charges for each item.

Reagents

Item	Quantity Needed	Preferred Vendor	Vendor Product Number	List Price / Quantity
HeLa Cell Culture	1	ATCC	CCL-2	$359.15 / culture
Gibco™ DMEM, High Glucose, Pyruvate	500 mL	Fisher Scientific	11-995-065	$22.90 / 500 mL
Gibco™ Fetal Bovine Serum	55 mL	Fisher Scientific	26-140-087	$162.00 / 100 mL
Gibco™	5 mL	Fisher Scientific	10-378-016	$28.05 / 100 mL

Penicillin-Streptomycin-Glutamine, 100X				
Gibco™ Phosphate Buffered Saline Solution, pH 7.4, 1X	200 mL / lab	Fisher Scientific	10-010-023	$19.41 / 500 mL
Gibco™ Trypsin-EDTA (0.05%), Phenol red	15 mL / lab	Fisher Scientific	25-300-054	$12.17 / 100 mL
Gibco™ Trypan Blue Solution, 0.4%	5 mL	Fisher Scientific	15-250-061	$15.84 / 100 mL
Dimethyl sulfoxide	Varies	Sigma Aldrich	D8418-50ML	$38.10 / 50 mL
Ethanol, Laboratory Grade, 95%	100 mL	Carolina Biological	861283	$26.50 / 3.8 L
Ethanol, Laboratory Grade, 70%	Varies	Carolina Biological	861263	$23.35 / 3.8 L
Sodium Chloride, Reagent Grade	10 g	Carolina Biological	888883	$20.20 / 2 kg
Potassium Chloride, ACS Grade	10 g	Carolina Biological	882910	$10.25 / 500 g
Sodium Fluoride, Laboratory Grade	10 g	Carolina Biological	889308	$10.05 / 100 g
Bleach, 10%	1.5 L / lab	Generic	N/A	N/A

Supplies

Item	Quantity Needed	Preferred Vendor	Vendor Product Number	List Price / Quantity
Spray Bottle	1	Generic	N/A	N/A
Unisex Lab Coats	1	Fisher Scientific	Varies	Varies
Small Robust™ Blue Nitrile Gloves	Varies	VWR	490010-852	$139.91 / 100
Medium	Varies	VWR	490010-854	$139.91 / 100

Robust™ Blue Nitrile Gloves				
Large Robust™ Blue Nitrile Gloves	Varies	VWR	490010-856	$139.91 / 100
X-Large Robust™ Blue Nitrile Gloves	Varies	VWR	490010-858	$139.91 / 100
CELLSTAR® Filter Cap Cell Culture Flasks, 75 cm^2	1	VWR	82050-856	$496.96 / 120
Falcon™ Standard Tissue Culture Dish, 100 x 20 mm	10	Fisher Scientific	08-772E	$315.00 / 200
5 mL Disposable Serological Pipets	Varies	VWR	89130-896	$110.02 / 200
10 mL Disposable Serological Pipets	Varies	VWR	89130-898	$118.99 / 200
25 mL Disposable Serological Pipets	Varies	VWR	89130-900	$257.30 / 200
Stainless Steel Pipet Sterilization Box	1	VWR	82027-604	$44.74 / Ea
Disposable Pasteur Pipets, Glass	Varies	VWR	14673-043	$165.95 / 1,000
GeneMate Sterile Centrifuge Tubes, 15 mL	Varies	VWR	490001-621	$331.60 / 500
15mL Conical Poxygrid™ Tube Rack	10	Fisher Scientific	14-791-6D	$47.25 / 1
GeneMate 2.0 mL Graduated Microcentrifuge Tubes	20	VWR	490004-458	$95.03 / 500

Polypropylene Microtube Storage Racks	10	Fisher Scientific	05-541-1	$53.98 / 5
Disposable Transfer Pipets, Graduated	Varies	VWR	414004-017	$50.32 / 500
KOVA™ Glasstic™ Slide 10 with Grids	40	Fisher Scientific	22-270-141	$144.45 / 100
GeneMate Racked Pipet Tips, 200 uL	10	VWR	490000-380	$80.56 / 10
GeneMate Racked Pipet Tips, 1000 uL	10	VWR	490000-394	$92.62 / 8
Black Permanent Markers	10	Generic	N/A	N/A
PolsarSafe™ Ice Pan, 7.5 l	2	VWR	10736-446	$42.80
General-Purpose Laboratory Labeling Tape	1	VWR	89097-986	$44.41 / 6
Glass Low-Form Griffin Beakers	10	Fisher Scientific	FB100400	$62.00 / 12
Poxygrid® Bench-Top Biohazard Bag Holder	10	VWR	11215-508	$31.82 / Ea
Biohazard Bags for Poxygrid® Bag Holder	10	VWR	11215-857	$24.29 / 100
SCIENCEWARE® Heavy-Duty Biohazard Disposal Bags	1	VWR	11215-866	$614.81 / 200

Equipment

Item	Quantity Needed	Preferred Vendor	Vendor Product Number	List Price / Quantity
CellGard ES (Energy Saver) NU-480 Class II, Type A2 Biosafety Cabinet	1	NuAire	N/A	Request a Quote
Argos Evac Waste Aspiration System, 4 L	1	VWR	89129-550	$2,689.63 / Ea
In-VitroCell ES (Energy Saver) NU-5800 Direct Heat CO_2 Incubator	1	NuAire	N/A	Request a Quote
CO_2 Regulator Two Stage	Varies	NuAire	NU-1564	$345.00 / Ea
CO_2 Cylinder	Varies	Airgas		Request a Quote
Leica DMi1 Inverted Microscope	1	Associated Microscope Services	N/A	Request a Quote
INTEGRA PIPETBOY acu 2 Pipet Aid	1	VWR	37001-860	$417.57 / Ea
Refrigerator, 4°C	1	Generic	N/A	N/A
Freezer, -20°C	1	Generic	N/A	N/A
Isotemp™ General Purpose Deluxe Water Bath	1	Fisher Scientific	S28124	$969.30 / Ea
VWR® Clinical 200 Large Capacity Centrifuge	1	VWR	82017-654	$3,084.09 / Ea
Leica DM500, Binocular Microscope	18	Associated Microscope Services	N/A	Request a Quote
Getinge 522LS Steam Sterilizer	1	Sterilink, Inc.	N/A	Request a Quote

Eagle™ Step-On Biohazard Waste Container	1	Fisher Scientific	18-880A	$132.00 / Ea
Dynalon™ Bio-Bin™ Waste Disposal Containers	1	Fisher Scientific	12-009-801	$215.80 / 40
Sharps-A-Gator™ Point-of-Use Sharps Containers	1	Fisher Scientific	14-827-129	$12.50 / Ea

HeLa Cell Culture Handling

Care of HeLa cell line

Vendors often ship cell lines frozen on dry ice in cryopreservation vials. When the cell line is received, the HeLa cells should be revived in a fresh culture following the vendor's recommended protocol or stored below -130° C in liquid nitrogen vapors. Failure to do so may compromise the integrity of the cell line and may interfere with the experimental results.

In order to minimize potential contamination to the cell line or any reagents, utilize aseptic techniques throughout preparation of HeLa cell cultures. These include but are not limited to:

a. Opening cryopreservation vials, centrifuge tubes, reagent bottles, culture flasks, petri dishes, serological pipettes and any other sterile materials only under a clean biosafety cabinet with its blowers running
b. Decontaminating all items or their sealed packaging by spraying them with 70% ethanol before placing them in a clean biosafety cabinet with its blowers running
    - This includes nitrile gloves the user should be wearing
c. Placing the cap or lid to every vial, centrifuge tube, reagent bottle, culture flask and petri dish back on its container whenever it is not being used
d. Preventing pipettes from contacting anything but reagents and the inside of culture dishes once they have been removed from their sterile wrapper or sterilization box
e. Ensuring that reagents are not pulled up into the cotton plug at the top of each serological pipette; placing all serological pipettes back into their wrappers after use
    - Avoid using the same serological or Pasteur pipette repeatedly

Disposal of contaminated materials

Discard all solid waste in the appropriate biohazardous waste container after use: plastic serological pipettes in a Dynalon™ Bio-Bin™ waste disposal container, glass Pasteur pipettes in a Sharps-A-Gator™ Point-of-Use sharps container, and any nitrile gloves, cryopreservation vials,

centrifuge tubes, culture flasks and petri dishes in an Eagle™ Step-On biohazard waste container lined with a biohazardous waste bag. Sterilize each sealed bag or container via autoclave at 121°C and 100 kPa above atmosphere pressure for 15 minutes. Waste can be discarded alongside other trash after decontamination.

Disinfect any aspirated liquid waste by adding enough bleach to the waste container to bring the final concentration of bleach to 10%. Expose the waste to bleach for at least one hour before flushing down the sink with plenty of water.

Safety Guidelines

This laboratory exercise should be conducted in accordance with your institution's chemical hygiene plan and / or biosafety plan. All students should have received laboratory safety training prior to participating in this exercise and must adhere to safe laboratory practices throughout the duration of the exercise. These practices include, but are not limited to:

- Wearing appropriate clothing and closed-toed shoes in the lab
- Wearing personal protective equipment and washing hands before leaving the lab
- Being familiar with the location and use of all the safety equipment in the lab
- Never eating, drinking, chewing gum or applying makeup while in the lab
- Finding out about a chemical's properties, hazards, and safety precautions before use
- Reporting any safety violations or unsafe conditions in the lab to instructor immediately

Supplemental Information

A brief introduction to HeLa cells, including information on their origin and their impact on subsequent biomedical research, is included in the *Student Handout*. Instructors may elect to compliment this laboratory exercise with other discussions or assignments on:

- Mitotic cell division, the cell cycle, and mutations leading to cancerous cell growth
- Excerpts from *The Immortal Life of Henrietta Lacks* by Rebecca Skloot (2010)
- Ethical questions surrounding the initial acquisition and subsequent use of HeLa cells
    - Particularly, the for-profit use of HeLa cells without a patient's consent

Laboratory Preparation

1. Read through both the *Instructor's Preparation Guide* and the *Student Handout* in their entirety to become familiar with the reagents, supplies and protocols that will be used.
2. **It may be necessary to submit a Biological Use Authorization (BUA) form for the HeLa cells used in this laboratory exercise to the Biosafety Committee or Environmental Health and Safety office at your institution. Confirm approval for the use of HeLa cells in this laboratory exercise at your institution prior to ordering or preparing any materials.**

Initiate Primary HeLa Cell Culture – 6 to 10 Days in Advance of the Exercise

The preparer should wear nitrile gloves, a lab coat and safety glasses, and should demonstrate safe and responsible laboratory practices throughout the preparation of the HeLa cell cultures. Proper use of a biosafety hood will not only help minimize the potential for contamination to any cultures and reagents, but also protect the user from biohazardous materials. The following protocol assumes the biosafety hood is outfitted with a waste aspiration system, an automated pipette aid, and racks that can accommodate a variety of centrifuge tubes.

1. **Prepare reagents, consumable supplies and the biosafety hood for use**
    a. Remove bottles of fetal bovine serum (FBS) and penicillin-streptomycin-glutamine from -20°C freezer and warm in 37°C water bath until at room temperature
    b. Remove bottle of Dulbecco's modified eagle medium (DMEM) from 4°C fridge and warm in 37°C water bath for approximately 10-15 min or until at room temperature
    c. Turn on the light inside biosafety hood, raise the shield to the appropriate height (as indicated on the side of the biosafety hood) and turn the blower on
    d. Spray the work area inside the biosafety hood with 70% EtOH and wipe clean
    e. Obtain each of the warmed reagent bottles, a sterile 75 cm^2 TC treated culture flask, a 15 mL centrifuge tube, and several 1 mL, 5 mL, 10 mL and 25 mL serological pipettes; spray each item with 70% EtOH and wipe clean before placing in hood

2. **Supplement DMEM with 10% FBS and 1% penicillin-streptomycin (100X)**
    a. Use serological pipettes to add 50 mL of sterile FBS and 5 mL of sterile penicillin-streptomycin-glutamate to a sterile bottle of 500 mL of DMEM; mix thoroughly
    b. Label the outside of the bottle of DMEM to reflect the amount of FBS and pen-strep

3. **Label the outside of the sterile 75 cm^2 TC treated culture flask with a permanent marker**
    a. Write the following information on the outside of the flask as small as possible
        i. HeLa (the cell type)
        ii. Your initials, the date, and the passage number (P1, P2, P3, etc.)
        iii. 1:10 (passage is performed using 1 mL of existing culture and 9 mL of media)

4. **Resuspend stock HeLa cell culture in a 75 cm^2 TC treated culture flask containing DMEM**
    a. Use a 10 mL serological pipette to add 9 mL of DMEM with 10% FBS / 1% Pen-Strep to the labeled 75 cm^2 TC treated flask; place the cap back on flask when not in use
    b. Thaw cryo vial of HeLa cells by warming in 37°C water bath for approximately 2 min
    c. Spray the vial with 70% Ethanol and wipe clean before placing it in the biosafety hood
    d. Transfer the contents of the vial to a sterile 15 mL centrifuge tube containing DMEM

i. The cryopreservation medium may contain dimethyl sulfoxide (DMSO), which will interfere with subculture cell growth. The greater the volume of DMEM added here, the greater any DMSO will be diluted and washed from the cells

e. Pellet the HeLa cells by centrifuging the 15 mL conical tube at 125 g for 10 minutes
   i. Use another 15 mL conical tube filled with water to balance the centrifuge
f. Spray the 15 mL conical tube with 70% Ethanol and wipe clean before placing in hood
g. Use a sterile glass Pasteur pipette to aspirate off the supernatant of DMEM without disturbing the pellet of HeLa cells adhered to the bottom of the 15 mL conical tube
   i. Place any used glass Pasteur pipettes in biohazardous sharps container
h. Use a 1 mL serological pipette to add 1 mL of DMEM with 10% FBS / 1% Pen-Strep to the 15 mL conical tube; pipette the suspension up and down repeatedly to mix
i. Use a 1 mL serological pipette to transfer the 1 mL of cell suspension to the labeled 75 cm^2 TC treated flask; rock the flask in an up-down-left-right motion to mix evenly

5. **Place HeLa culture flask in a CO_2 incubator and clean-up the biosafety hood**
   a. Lay the newly passed HeLa cell culture flat in a CO_2 incubator (at 37°C with 5% CO_2)
   b. Return bottle of DMEM with supplements to the fridge; any remaining FBS and Penicillin-Streptomycin should be aliquoted into smaller tubes to prevent superfluous freeze-thaw cycles from damaging the integrity of the reagents
   c. Discard all solid waste in the appropriate biohazardous waste container after use
   d. Spray the work area inside the biosafety hood with 70% Ethanol and wipe clean
   e. Turn the blower off, lower the shield, and turn off the light inside the biosafety hood

Pass HeLa Cell Subcultures in Dishes – 3 to 5 Days in Advance of the Exercise

This exercise was originally designed for a single class of 18 students working in pairs. If preparing subcultures for multiple laboratory sections, consider initiating the primary HeLa cell culture 9 to 15 days in advance of the exercise, passing the first subcultures in multiple flasks 6 to 10 days in advance, and then passing them in petri dishes 3 to 5 days in advance. Each 75 cm^2 flask containing 10 mL of active HeLa cell culture can be passed 1:10 into 10 petri dishes. Scale the number of subcultures prepared accordingly.

1. **Determine the confluency of HeLa cells using an inverted compound microscope**
   a. The percent confluency describes the amount of available space in the culture flask or dish that is covered by the dividing cells. This can range from an empty culture up to a saturated culture covering 100% of the treated surface in the container
   b. Proceed with passing cultures once the primary culture has reached 80% confluency
      i. Note - HeLa cells replicate approximately every 24 hours

2. **Prepare reagents, consumable supplies and the biosafety hood for use**
    a. Remove bottle of 0.05% trypsin-EDTA from -20°C freezer and warm in 37°C water bath until at room temperature
    b. Remove bottle of Dulbecco's modified eagle medium (DMEM) from 4°C fridge and warm in 37°C water bath for approximately 10-15 min or until at room temperature
    c. Turn on the light inside biosafety hood, raise the shield to the appropriate height (as indicated on the side of the biosafety hood) and turn the blower on
    d. Spray the work area inside the biosafety hood with 70% Ethanol and wipe clean
    e. Obtain each of the warmed reagent bottles, a sleeve of TC treated petri dishes, a 15 mL centrifuge tube, and several 1mL, 5 mL, 10 mL and 25 mL serological pipettes; spray each item with 70% Ethanol and wipe clean before placing in hood

3. **Label the lids of the sterile TC treated petri dishes with a permanent marker**
    a. Write the following information on the lid of the petri dish as small as possible
        i. HeLa (the cell type)
        ii. Your initials, the date, and the passage number (P1, P2, P3, etc.)
        iii. 1:10 (passage is performed using 1 mL of existing culture and 9 mL of media)

4. **Pass HeLa cell subcultures 1:10 in TC treated petri dishes containing DMEM**
    a. Use a 25mL serological pipette to add 9 mL of DMEM with 10% FBS / 1% Pen-Strep to the labeled TC treated petri dishes; place the lids back on the dishes when not in use
    b. Use a sterile glass Pasteur pipette to aspirate off the DMEM without disturbing the HeLa cells adhered to the bottom of the 75 cm^2 TC treated culture flask
        i. Tilt the culture flask to the side to pool the media in one of the corners
        ii. Place any used glass Pasteur pipettes in biohazardous sharps container
    c. Use a 10 mL serological pipette to add 10 mL of 1X phosphate buffered saline (PBS) to the flask; rock the flask in an up-down-left-right motion to wash the cells thoroughly
        i. FBS in the DMEM will neutralize the Trypsin used later to release cells if it is not washed thoroughly from the HeLa cells and surface of the culture flask
    d. Use a sterile glass Pasteur pipette to aspirate off the 1X PBS without disturbing the HeLa cells adhered to the bottom of the 75 cm^2 TC treated culture flask
    e. Use a 1 mL serological pipette to add 1 mL of 0.05% trypsin-EDTA to the labeled 75 cm^2 TC treated flask; rock the flask in an up-down-left-right motion to spread evenly
        i. Trypsin works to release the adherent HeLa cells from the flask surface
        ii. Incubate the flask at 37°C with 5% CO_2 for 5-10 min to optimize separation
        iii. Confirm adequate separation of cells from bottom of dish via microscope
            1. Cells should look more spherical than triangular once separated
    f. Use a 10 mL serological pipette to add 9 mL of DMEM with 10% FBS / 1% Pen-Strep to the 1 mL of 0.05% trypsin-EDTA already in the labeled 75 cm^2 TC treated flask
        i. Rock the flask in an up-down-left-right motion to mix evenly

g. Use a 10 mL serological pipette to transfer 1 mL of culture to each new dish

5. **Place HeLa culture petri dishes in a CO_2 incubator and clean-up the biosafety hood**
   a. Stack the newly passed HeLa cell cultures in a CO_2 incubator (at 37°C with 5% CO_2)
   b. Return bottles of DMEM to the fridge and Trypsin to the freezer; 10 mL of 0.05% Trypsin-EDTA should be aliquoted into a 15 mL tube for use during the exercise
   c. Discard all solid waste in the appropriate biohazardous waste container after use
   d. Spray the work area inside the biosafety hood with 70% EtOH and wipe clean
   e. Turn the blower off, lower the shield, and turn off the light inside the biosafety hood

Set Out Supplies – Day of the Exercise

1. **Provide copies of the *Student Handout* for each student**

2. **Aliquot out the following reagents in tubes for each student pair**
   a. 15 mL of 1X PBS in 15 mL centrifuge tubes (store in 37°C water bath)
   b. 1 mL of 0.05% Trypsin-EDTA in 2.0 mL microcentrifuge tubes (on ice)
   c. 1 mL of 1% FBS in 1X PBS in 2.0 mL microcentrifuge tubes (on ice)
   d. 250 uL of 0.4% Trypan Blue in 2.0 mL microcentrifuge tubes (room temp)

3. **Prepare stock solutions for use as potential treatments (biology majors only)**
   a. Prepare 1 M NaCl solution by dissolving 5.844 g of NaCl in 100 mL of 1X PBS
   b. Prepare 1 M KCl solution by dissolving 7.454 g of KCl in 100 mL of 1X PBS
   c. Prepare 1 M NaF solution by dissolving 4.198 g of NaF in 100 mL of 1X PBS
   d. Prepare 50% EtOH solution by mixing 52.6 mL of 95% EtOH in 47.4 mL of 1X PBS
   e. Provide enough of 1X PBS for use as a negative control throughout
   f. Sterilize each solution via autoclave at 121°C and 100 kPa for 15 minutes

4. **Place the following materials at every station for each student pair**

2 DM500 compound microscopes	4 KOVA Glasstic® slide 10 with grids
1 P20 micropipette and 20 uL tip box	10 graduated transfer bulb pipettes*
20 2.0 mL microcentrifuge tubes	1 microcentrifuge tube rack
1 15 mL centrifuge tube rack	1 ultra-fine-tip permanent marker
1 biohazardous waste stand with bag	1 labeled beaker with 10% bleach

*P1000 micropipettes can be used in lieu of disposable transfer bulb pipettes

5. **Set a refrigerator to 4°C; set water baths / gravity convection incubators to 37°C and 42°C**

6. **Take the HeLa cell culture dishes out of the CO_2 incubator when the lab exercise begins**
   a. Wear nitrile gloves and spray them with 70% EtOH before accessing the incubator

Instructor's Procedure

General Education Biology (non-majors)

1. Provide a brief introduction for the students to HeLa cells, including information on their origin and their impact on subsequent biomedical research
2. Discuss the anticipated timeline for the laboratory exercise, including the generation of the figure
3. Review laboratory safety guidelines prior to beginning the exercise, including the correct use of personal protective equipment as well as protocols for proper disposal of both solid and liquid waste
4. Assist students as they disperse the monolayer of HeLa cells from their petri culture dish and prepare replicate cell suspensions for each treatment. Remind students to properly label each tube
5. Assist students as they incubate their replicate HeLa cell suspension tubes at 4°C, 37°, or 42°C
6. Demonstrate how to use a KOVA Glasstic® slide 10 with grids and trypan blue to count living cells
7. Lead further discussion on the history of HeLa cells and the ethics surrounding their use in research
8. Assist students as they retrieve their replicates from each incubator and determine cell counts
9. Review the expectations for the graph and figure legend the students will generate outside of class

Biology Majors

1. Provide a brief introduction for the students to HeLa cells, including information on their origin and their impact on subsequent biomedical research
2. Discuss the anticipated timeline for the laboratory exercise, including the generation of the figure
3. Review laboratory safety guidelines prior to beginning the exercise, including the correct use of personal protective equipment as well as protocols for proper disposal of both solid and liquid waste
4. Approve the experimental design for each pair of students, ensuring that they have a negative control, three appropriate treatment groups, and three replicates for each
5. Demonstrate how to use a KOVA Glasstic® slide 10 with grids and trypan blue to count living cells
6. Assist students as they disperse the monolayer of HeLa cells from their petri culture dish and prepare replicate cell suspensions for each treatment. Remind students to properly label each tube
7. Assist students as they initiate the treatments for each experimental group
8. Lead further discussion on the history of HeLa cells and the ethics surrounding their use in research
9. Assist students as they sample replicates in each experimental group and determine cell counts
10. Review the expectations for the graph and figure legend the students will generate outside of class

Table 1a-1b: Student Feedback from HeLa Cell Laboratory

Table 1a: Feedback from Non-Biology Majors
What made this lab difficult?
• I felt inexperienced working in the lab. • The protocol was too long and extensive. • The calculations were very involved and confusing. • Cell counting was really hard, especially when they were concentrated. • This lab was more complex than previous labs. • The procedure was hard to understand. • Inexperienced interpreting scientific terminology
What did you learn from this lab about human cells?
• How cells look under the microscope. • Cell culture is delicate and requires a lot of patience. • I learned how to distinguish live and dead cells. • Ethics of working with mammalian cells • How to manipulate HeLa cells for scientific research • How to remove cells from the bottom of a plate using trypsin. • The best conditions for cells to survive in the lab is the same as the human body.
In general, what did you learn about this lab?
• Cell culture protocols can be extensive. • Cell viability changes based on different treatments. • How to analyze data in Excel. • I learned that precision is essential for accurate results. • Ethics of scientific research. • I learned about the history of HeLa cells, where they originated and why they are important. • Mammalian cells are complex. • I learned how to use the lab equipment like pipettes and the microscope. • How to tell the difference between live and dead cells under the microscope.
What did you like about this lab?
• Looking at mammalian cells under the microscope. • It was interesting to learn about the history of HeLa cells and Henrietta Lacks. • I liked the hands-on experience of working with cells. • The homework helped to understand the material. • Clear and easy to follow instructions. • The professor really helped to make the lab easier to understand. • Learning about cells and how they pertain to humans. • The assignment instructions were easy to understand. • I liked that we were able to work in groups. • It was really rewarding to see results turn out. • I was excited to work with real mammalian cells. • We had independence with using reagents. • It was really different from the other labs we have done. • Forming a hypothesis and testing it. • The material presented was comprehensible and it was clear why the material is important.
What did you *dislike* about this lab?

• We finished past the allotted time, which made me late for my next class. • The instructions were very long and confusing. • It was difficult to use and adjust the microscope. • Counting cells was very long. • There was a lot of scientific language I did not understand. • You had to be very precise with the measurements. • We had to use so many different reagents. • There was too much downtime during the incubations. • The calculations were confusing. • Even though we worked really hard in this lab, we did not get good results. • The class spent too much time on data analysis.
What would make the experience better for you?
• If we were able to finish in time. • I would have liked it if we used our downtime for educational purposes. • It would have been better with fewer calculations. • Easier and more detailed instructions. • If the pre-lab reading assignment was shorter. • I think we should have split the lab into 2 classes. • More visual examples. • More relevant prelab readings. • I would have liked more help in data analysis**.** • Knowing more about HeLa cells before starting the lab to make it clearer.

Six instructors collected student feedback outlined in the table above. To avoid repetition, one representative comment was presented on this table when student comments were similar.

Table 1b: Feedback from Biology Majors
What made this lab difficult?
• Counting the cells was somewhat difficult due to the fact that it was hard to tell the difference between live and dead cells. • The amount of accuracy and preparation for the lab was the most difficult part. • The data analysis was complicated. • The protocol was very long and confusing. • It was hard to figure out what to do for my experiment and find my hypothesis. • It was hard to do the calculations and figure out the ratios to make the solutions. • Pipetting the solutions. • It was hard to use the microscope and I had trouble finding the cells. • It was not that hard. • The instructions were not clear and there were a lot of steps. • The time constraint made it hard to get everything done. • Keeping track of all the microcentrifuge tubes.
What did you learn from this lab about human cells?
• The effects of treating cells and that they can easily be killed by different things likes temperature, pH or difference salts. • Cell are incredibly sensitive to different environments and can't survive in certain environments or conditions. • That cells can be counted. • I learned that living human cells can be seen under a microscope. • Lab experience is required to work with mammalian cells. • About the background of HeLa cells and who they came from.

- Cancerous cells can replicate forever and become immortal.
- I learned that cells are easily manipulated by different procedures done to them.
- That they are hard to work with.
- I learned that alcohol kills human cells.
- I learned that trypsin is needed to remove cells from the bottom of the plate. I did not realize that they were attached and formed a bond that needs to be broken.

In general, what did you learn about this lab?

- I found out that cells respond to external stimuli.
- That difference treatments influence whether cells live or die.
- How to count cells with a microscope.
- I learned how to create different cell concentrations and how to tell the difference between live and dead cells.
- How to use trypsin to move cells to another container.
- How to use and adjust a microscope properly.

What did you like about this lab?

- I really enjoyed that we could customize our own protocol and choose our treatment.
- I like that we could see the cells under the microscope.
- Using and adjusting the microscope.
- It was nice to use the scientific method to develop our experiments.
- I liked to use the formula to count our cells.
- Seeing the results after hard work.
- I liked that mammalian cells come from "us", making the lab more relatable

What did you *dislike* about this lab?

- Counting the cells was hard and took a long time. My eyes started to hurt after a while.
- The class was way too long and we did not have enough time to finish the experiments.
- The data analysis was challenging and we had to use Excel.
- The complexity/difficulty of this lab.
- Unclear/hard to interpret the instructions.
- There was too much downtime while we were waiting for the cells.
- I didn't see any pattern in my results.
- It was difficult to collect reliable data from my cells.
- There didn't seem to be a purpose to this lab.
- I hated the dilution calculations to figure out how much ethanol we had to add.

What would make the experience better for you?

- Better cooperation from my lab partner.
- I think we should have split up the class to preform identical experiments and compare then results.
- It would have been easier if I had been better prepared before the lab.
- Better explanation of the purpose of the lab and the experiments.
- Use one class just to teach excel and how to use the microscope.
- I would have liked pictures and demos to grasp the material.
- Split the lab into 2 classes, the experiment was too long in 1 class.

Six instructors collected student feedback outlined in the table above. To avoid repetition, one representative comment was presented on this table when student comments were similar.